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Progress Report

RESISTANCE OF PINES TO BARK BEETLES

Studies on Toxicity of Resins

1960

By Richard H. Smith, Entomologist

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U. S. DEPARTMENT OF AGRICULTURE, FOREST SERVICE PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION

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# U. S. DEPARTMENT OF AGRICULTURE - FOREST SERVICE PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION Division of Forest Insect Research

Progress Report

RESISTANCE OF PINES TO BARK BEETLES

STUDIES ON TOXICITY OF RESINS

1960

By Richard H. Smith

#### SUMMARY

Research on the resistance of pines to bark beetles was continued in 1960 at the Institute of Forest Genetics, Placerville, California. Again the emphasis was on the toxicity of pine resin vapors to adult <u>Dendroctonus</u> beetles. The major objectives were: (1) to test the hypothesis that the beetles can tolerate saturated vapors of host pines but cannot tolerate the vapors of nonhost pines, (2) to investigate local and seasonal variations in beetle and resin characteristics, (3) to test the resins of pine hybrids against the beetles.

Three species of Dendroctonus beetles served as test insects:

D. monticolae Hopk., D. jeffreyi Hopk., and D. brevicomis Lec. The beetles were reared to the adult stage from infested material gathered from forests of the central Sierra Nevada and held in an insectary at the Institute. Adults were collected within one day after they emerged and either used immediately or held at 35° F. for a day or two prior to their use. The resin materials tested were from six species of hard pines, four hard-pine hybrids, and two soft pines. Resin was obtained with a micro-tapping device from these trees growing at the Institute. Testing techniques developed in previous studies were used.

Thirty-four separate experiments, each involving two or more resins and a control, were conducted. The average experiment was composed of four treatments plus the control, each consisting of six replicates of 10 to 12 beetles apiece. Beetles were assigned to replicates equitably by sizes, and replicates were randomly assigned to the various treatments. Conditions under which the tests were conducted and the mortality readings made were standardized.

Beetle mortality data from each test were subjected to an analysis of variance. Where the treatment F-value was significant at the 90 percent confidence level, the data were then subjected to Duncan's multiple-range testing. Confidence lines were drawn at both the 95 and 99 percent level to determine significantly different treatments.

Twenty-eight of the experiments were made with the resin vapors at saturation, and the other six with a vapor concentration at approximately 50 percent of saturation. The concentration of vapor present in the fumigation chamber was determined by weighing representative samples of resin in each test.

The tests showed that in all of the hard pines which were natural hosts of the beetles the resin vapors were not significantly toxic. But in ten of the eleven cases involving hard pines which were not natural hosts, the resin vapors were significantly toxic. The one exception, P. sabiniana with D. jeffreyi, may be explainable on the basis of resin chemistry.

Results with hybrids of the hard-pine group show that (a) none of the three beetles could tolerate saturated resin vapors of a nonhost x nonhost hybrid (P. attenuata x radiata) and (b) resin vapors of nonhost x host hybrids were generally toxic to D. brevicomis and D. jeffreyi but nontoxic to D. monticolae.

The vapor toxicity of the soft-pine resins to the beetles tested did not show the same consistency with beetle-host relationships as it did with the hard pines. D. jeffreyi was the only species affected by the resin vapors of soft pines, and then only to a limited degree during the posttreatment period.

Resins from P. ponderosa representing widely separated geographic sources did not vary significantly in their toxicity to D. brevicomis. Significant differences were sometimes obtained between individual P. jeffreyi x ponderosa hybrids with different maternal parents.

No differences were found between the fresh resin of ponderosa pine and its supernatant liquid, but significant differences were found between fresh resin and turpentine, the heat-fractionated derivative of resin. There were wide variations in percent volatility and saturation weights of resin vapor among the trees tested. These characteristics were not generally associated with the toxicity of resin vapor.

The results of several experiments covering a 2- to 3-month period strongly suggest that seasonal variations occur in the vigor of D. brevicomis as measured by the rate of natural mortality and the quality of P. ponderosa resin as measured by changes in vapor saturation weight.

#### INTRODUCTION

The role of pine resin in the success or failure of bark beetle attacks has received serious attention for the past several years in pursuing the basic question of the resistance of pines to bark beetles. Our research in three previous seasons has been devoted largely to the question of the toxicity of pine resins to bark beetles (Smith, 1961a, 1961b). Though various aspects of toxicity have been explored, toxicity of resin vapors to adult bark beetles has been found to lend itself most readily to research techniques. The studies thus far have produced results that are believed to most closely approximate basic relationships between the genus Dendroctonus and the genus Pinus.

The results of this research have both academic and practical importance. Academically they contribute toward a better understanding of the relationship between bark beetles and their hosts. Also, they help to shed light on seasonal or local variations in beetles and resins. Craighead et al. (1931), in reviewing bark beetle control, recognized the need for such knowledge many years ago. They concluded (p. 1,002) that ". . . the problem (of bark beetle control) failed to take into account the complex biotic factors which control the abundance of insect populations and govern the rise and fall of bark beetle epidemics. These even now after many years' experience in control and investigations are very little understood." Though our understanding may have increased since then, a great many facets exist in the relationship which cannot be satisfactorily explained. Bark beetles are not unique among insects, yet they do have certain unusual ecological relationships; for example, they attack a living host but only the rapid death of the host insures a successful brood.

Several practical ends are served by this work: (1) a better understanding and improvement of the ponderosa pine risk-rating system; (2) a more positive approach in silviculture which will ultimately permit insect-resistant trees to be retained rather than susceptible ones eliminated as is now the case; (3) knowledge of criteria needed to guide forest geneticists in selecting desirable genotypes for tree breeding; and (4) development of measures for determining the relationships between pine bark beetles and pine hybrids.

This report contains a brief review of previous investigations conducted by federal workers in California on the subject of resistance of pines to bark beetles. Special attention has been given to the studies of the past 10 years.

The report covers in detail the research conducted in 1960 on toxicity of pine resin to bark beetles. The scope of this work was enlarged during the year to include three species of Dendroctonus bark beetles and several species of pines and pine hybrids. Of these beetles, D. jeffreyi is monophagous on Pinus jeffreyi; D. brevicomis is oligophagous on P. ponderosa and P. coulteri; D. monticolae is polyphagous on P. lambertiana, P. monticola, P. ponderosa, P. coulteri, and P. murrayana.

The pines were selected to include hosts and nonhosts of each beetle and hybrids of hosts and nonhosts.

The 1960 field work was again carried on at the Institute of Forest Genetics, Placerville, California. Certain examinations and all the data analyses were made in Berkeley. The Institute's facilities were made available for the work and upon occasion personnel of Division of Forest Genetics helped out as needed. Melvin D. Sage assisted in collecting the field data. Richard W. Bushing assisted in the laboratory examinations and data analysis during the winter.

An appendix of basic data, because of its bulk, is held in Berkeley.

#### REVIEW OF PREVIOUS WORK

In discussions of resistance or susceptibility of pines to bark beetles, some association between resins and bark beetle species attacking pines is often implied. These implications are rarely supported with facts. Sometimes, as with <u>D. valens Lec.</u> and <u>D. terebrans Oliv.</u>, the sentiment leans toward resin acting as an attractant. Other times, as with <u>D. brevicomis Lec.</u> and <u>D. monticolae Hopk.</u>, the feeling is toward an adverse effect of resin.

One of the first efforts to determine a specific relationship between D. brevicomis and ponderosa pine (P. ponderosa Laws.) resin was made by Gordon (1932). He concluded that turpenes, fractionated derivatives of resin, were toxic to adult beetles. Shortly before this work, H. L. Person and N. T. Mirov gave some consideration to the problem. Though not thinking of the direct association like Gordon, they did associate resin flow with the failure of bark beetle attack. In discussing D. brevicomis, Person (1926, p. 12) states: "The theory has been advanced that the beetles attack indiscriminately but are able to kill only the slow-growing trees which offer the least resistance in the way of resin flow. This view is hardly tenable since in spite of the great amount of cruising that is done very few trees are found which have been unsuccessfully attacked. At certain times, however, when the number of beetles on an area is too great for the number of slow-growing trees the resistance of the fast-growing trees is an important factor in reducing the infestation. A much larger number of beetles are required to overcome the resin flow of the fastgrowing trees and this concentration often causes crowding of galleries and a consequent high brood mortality." Person (1931) later proposed a theory to explain host selection by D. brevicomis.

The problem of beetle-host relationships received only minor and cursory consideration until 1950 when J. M. Miller initiated additional experiments. The work that he started in this field has been continued with few interruptions for 10 years. Early in 1950, discussions were held at the Institute of Forest Genetics to consider the question of the resistance of pines to bark beetles, particularly the resistance of pine hybrids which had been developed by the Institute. In this discussion the possible role of turpenes was mentioned. Miller (1950) first undertook forced-attack studies which indicated that D. jeffreyi appeared to be unable to attack the Jeffrey x ponderosa pine hybrid. He also found that D. brevicomis was unable to attack Jeffrey pine (P. jeffreyi Grev. & Balf.).

In 1951 Miller assisted by R. Z. Callaham expanded these forced-attack studies. It was found (Callaham and Miller, 1952) that: (1) D. brevicomis, D. monticolae, and D. jeffreyi, were able to maintain prolonged attacks on their natural hosts, though no broods resulted from the attacks; (2) D. jeffreyi and D. monticolae were able to attack the Jeffrey x ponderosa hybrid, though the attacks were not successful; (3) hybrid pines had a gradation in resin characteristics ranging from

a position near one parent to a position near the other parent; (4) the larger beetles of D. monticolae were able to persist longer than the smaller beetles in their attacks on both host and nonhost pines. In summarizing their ideas, Callaham and Miller (1952, p. 6) state in part ". . . one of the factors which account for the host specificity of a given insect species is its ability to tolerate the oleoresin of certain pine species. \* \* \*. These tests also indicate that when an insect can tolerate the oleoresin of a given pine, then the quantity of resin produced by individual trees becomes a critical factor in the success or failure of an attack." Callaham (1952a) speculated upon both the physiological and anatomical basis for resin flow, and suggested the possible role of resin pressure in bark beetle success or failure. He also noted the relationship between resin pressure and moisture tension. In a separate report, Callaham (1952b) proposed a theory in explanation of host selection and host susceptibility in the western pine beetleponderosa pine complex. He states in part (p. 7) "...that attack by the first emerging adults of any generation is at random, and that the success or failure of these attacks is determined by the quantitative resin flow of the trees which are attacked. Attacks in resistant trees would be 'pitched out,' and no secondary attraction would be initiated. On the other hand, successful attacks in susceptible trees would result in a secondary fermentative attraction which has been described by Person. This attraction would result in a concentrated attack on the susceptible trees by beetles emerging during the remainder of the generation."

In the forced-attack studies conducted with other pines and pine hybrids, Callaham (1953a) found that both western pine beetle and mountain pine beetle were able to persist longer on host than on nonhost pines as a general rule. But neither beetle was able to produce broods even in its natural hosts under the conditions of the tests. The results were difficult to assess since most of the data were suitable for only subjective analysis. The lack of a testing technique that would produce more objective data led to abandonment of forced-attack studies.

The toxicity of resin to bark beetles and the possibility that turpenes in resin might act in this capacity were considered by Callaham (1953b). Early work by Gordon had shown that pinenes were toxic to western pine beetle. These leads were not pursued, however. Instead research on resistance was shifted to studies of the quantitative flow of resin in relation to bark beetle attack (Callaham, 1955). Resin flow from ponderosa pines of different insect risk classes denoting different degrees of susceptibility to bark beetle attack (Salman and Bongberg, 1942) was investigated. One major conclusion was that within three days after wounding, the flow of resin virtually ceased from high-risk trees, whereas there was a continued flow of resin from low-risk trees after three days. Callaham considered that the length of time a tree could produce resin helped to explain success or failure of bark beetle attacks. Early cessation of the flow from high-risk trees permitted the beetles to continue feeding, oviposit, and develop successfully; continued flow from low-risk trees prevented feeding and oviposition.

In 1956, when the author was assigned to this project, the work was reoriented to study the toxic effect of pine resins on bark beetles. In the period since then, investigations have been focused upon this subject, including contact, stomach, and fumigant toxicity. As previously noted, only fumigant toxicity has been investigated in detail. Much effort has been required to develop suitable techniques for testing the toxicity of pine resin vapors (Smith, 1959a). This problem was resolved, however. Research on fumigant toxicity that has been conducted thus far indicate that beetles can tolerate saturated resin vapors of host trees but cannot tolerate those of nonhost trees (Smith 1959b, 1959c).

#### PROCEDURES FOR STUDYING RESIN VAPOR TOXICITY

Certain standard procedures were used in all tests carried out in 1960 for determining the toxicity of pine resin vapors to adult bark beetles. These procedures involved (a) collecting and handling beetles, (b) tapping trees for resin, (c) establishing experiments and collecting data, and (d) analyzing data. They apply to all tests described in this report unless otherwise noted.

#### Collecting and handling beetles

The three species of beetles used were reared at the Institute from naturally infested trees cut from various forest locations in the central Sierra Nevada (figure 1). Trees infested with D. monticolae or D. jeffreyi were cut into 18-inch lengths and the bolts were taken to the laboratory. Trees infested with D. brevicomis were felled and debarked or were debarked without felling, and only the bark was taken. Sometimes the infested material was moved to an insectary where the beetles could mature and emerge under the prevailing summer weather at the Institute. At other times, when the beetles were not needed immediately, it was placed in a coldroom at 35° F. for a period of time and later moved to the insectary. Each day at approximately 8:00 a.m. and 8:00 p.m. beetles which had emerged from the brood material in the insectary were collected individually in #000 gelatin capsules and held at 35° F. until a sufficient number had been collected for a test. With the exception of a few tests with D. jeffreyi, collected beetles were not held for more than three days.

Dendroctonus monticolae: Approximately three cords of ponderosa pine containing overwintering broods were cut on June 16 and 17 at Crystal Bay, Nevada. The trees ranged from 12 to 20 inches d.b.h. At the time they were cut the brood development varied from half-grown larvae to callow adults. The brood material was first placed in a basement at the Institute, where fairly normal indoor temperatures prevailed. On June 23, the bolts were transferred to the insectary. On July 1, approximately one more cord was cut at the same location and added to the material already in the insectary. The daily emergence of beetles from this material is shown in figure 2. Although the bolts contained mostly D. monticolae, some of them had an intermingling brood of

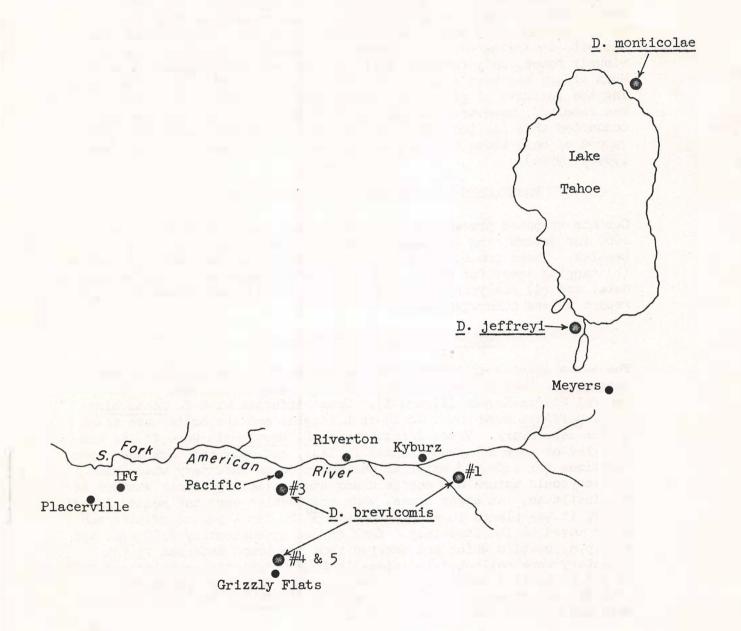


Figure 1.--Sources of <u>Dendroctonus</u> broods used in vapor toxicity tests; <u>D. brevicomis</u> brood #2 was obtained near Bass Lake in Madera County.

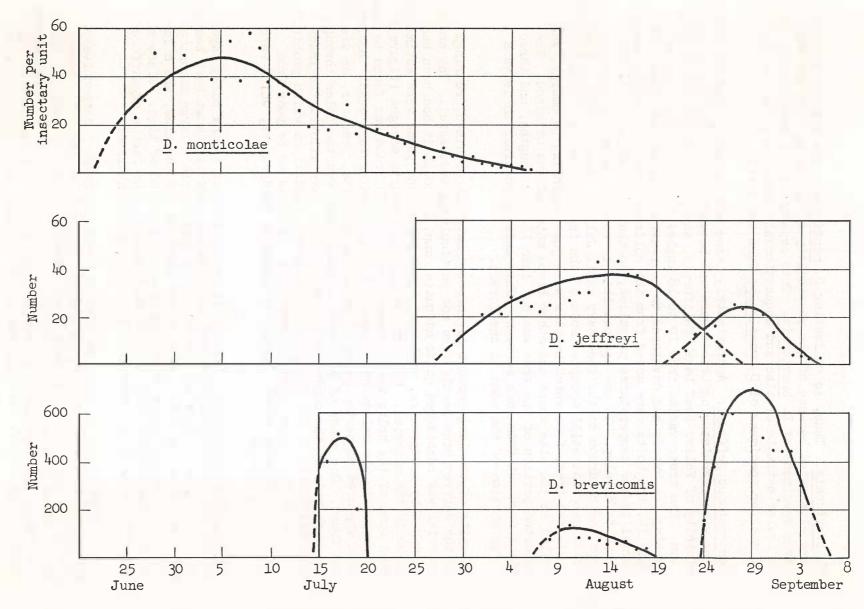


Figure 2.--Emergence of three species of <u>Dendroctonus</u> from brood material in insectary at Institute of Forest Genetics.

 $\underline{D}$ .  $\underline{brevicomis}$ . Thus, in collecting  $\underline{D}$ .  $\underline{monticolae}$ , the smaller beetles were eliminated because of the possibility of mistaking them for very large specimens of  $\underline{D}$ .  $\underline{brevicomis}$ . This separation was based solely on an ocular estimate of size and unquestionably resulted in the exclusion of smaller specimens of  $\underline{D}$ .  $\underline{monticolae}$  from the tests.

Dendroctonus jeffreyi: Approximately three and one-half cords of Jeffrey pine with overwintering broods were cut between June 10 and 15 in the vicinity of Fallen Leaf Lake, California, and placed in the 35° F. coldroom. The trees varied from 10 to 18 inches d.b.h. At the time of cutting the broods had developed to near-mature larvae and pupae. On July 14, the bolts were moved from the coldroom into a basement, where normal inside temperatures prevailed, so that the insects could continue to develop. It was not possible to move the material directly from the coldroom to the insectary until July 25, because the D. monticolae brood material still occupied most of the insectary space. On August 11, a 35-inch tree, from which beetles were just beginning to emerge, was felled at the site where the other brood material was obtained. The infested portion of the tree was cut into 18-inch lengths, and three slabs were cut from each section. This made it possible to leave a large portion of the wood in the forest.

In spite of the large amount of infested material obtained, rearings of the Jeffrey pine beetle were not particularly successful. The brood material was considered quite adequate, and, although it had been heavily attacked by woodpeckers, well over 6,000 larvae and pupae were estimated to be in the material. However, barely 1,000 adults emerged (figure 2). When some of the bolts were examined, most of the brood was found to have developed to the callow adult stage, but the beetles were dead. Desiccation appeared to be the cause of death; no parasite, predator, or disease was found. One cause of desiccation could have been the holes through the bark made by the woodpeckers. These might have allowed the inner bark and phloem to dry out rapidly and create conditions which prohibit successful brood development. Emergence was found to be poor from similarly infested trees, heavily attacked by woodpeckers, which were left in the forest.

Dendroctonus brevicomis: Since this beetle matures in the corky layers of the bark, rather than between the wood and bark like most bark beetles, only the infested bark was needed. With this beetle a large amount of brood material could be placed in the insectary at one time, and a large number of beetles could be reared in a short period of time. Since this species, unlike the other two, is multivoltine, the factor of different generations could be introduced into the experimentation.

Five different collections, designated brood 1 through 5 respectively, and all from ponderosa pine, were made. Brood #1 of the first summer generation was obtained on July 8 and immediately placed into the insectary. Brood #2 was the overwintering beetles which were in bark removed from trees near Bass Lake, Sierra National Forest, in late April. This material was held in the coldroom at  $35^{\circ}$  F. until July 20, when it was

transferred to the insectary. Brood #3, obtained on July 26, was either late first generation or an early second generation. It was held in the coldroom at 35° F. until August 2, when it was moved to the insectary. Brood #4, either late second generation or an early third, was obtained August 17 and placed directly in the insectary. Brood #5 was obtained a week later from the same area as #4. It also was moved directly into the insectary. Figure 2 gives a generalized picture of the emergence of three of the broods.

## Collecting Resins

All resins were obtained from trees growing at the Institute of Forest Genetics. Table 1 lists these trees, along with data pertaining to their identity, location within the Institute grounds, age, and diameter. All trees except the local California ponderosa pine were planted and occupy a specific grid location at the Institute. The local ponderosa pine is one of many growing wild there.

Resin was obtained by a micro-tapping device. A punch wound, one and one-half inches in diameter, was made through the bark and at least one-quarter inch into the sapwood. The disk of bark and wood was removed and a tubular plastic tap was fitted into the wound. A 30 cc. glass vial was attached to an opening on the lower surface of the plastic tap. The resin flowed from the wound into the plastic tap and down into the glass collecting vial. The collecting vial could be easily replaced without disturbing the remainder of the tap. To insure sufficient fresh resin for an experiment, trees were tapped six to forty-eight hours before the experiment was started.

# Setting up an Experiment

Experiments to test the vapor toxicity of resins were set up in much the same manner as that described by Smith (1961a). The beetles, still in cold storage and in individual gelatin capsules, were equitably distributed by size (ocularly estimated) among all the replicates in a test. Equalization of size reduced to a minimum the bias which size introduced into the rate of natural mortality. The replicates were randomly assigned to the treatments in a test. They were assembled at  $73^{\circ} \pm 2^{\circ}$  F. by transferring each beetle from a gelatin capsule to a fumigation cell. Less than one minute was required to assemble a replicate.

Two types of cells were used. For <u>D</u>. brevicomis the cell was a l-inch piece of 5 mm. i.d. glass tubing, four lengths of which were bound together into a bundle, with a piece of lumite screening fastened to one end. Nylon thread was used for all binding. A replicate of <u>D</u>. brevicomis consisted of three bundles of four cells each. After the beetles of a replicate had been transferred from the gelatin capsules to the cells, the three bundles were bound together into a stack. The bottom of each bundle served as a cap to contain the beetles in the cells of the bundle beneath it. The openings of the cells of the top

Table 1.--Name, location, age, and size of pines at the Institute of Forest Genetics which served as a source of fresh resin for conducting resin vapor toxicity tests.

Scientific name <sup>1</sup> / <u>Pinus</u>	: Common name <sup>2</sup> /	: Locat : Row :	ion <u>3/:</u> Tier:	Age4/	D.B.H.
	Hard pines			Years	Inches
ponderosa Laws	California ponderosa		-	40	30.0
ponderosa Laws.	Washington ponderosa	72	35	32	10.2
ponderosa var. arizonica (Engelm.)Shaw		67	27	30	11.0
ponderosa var. scopulorum Engelm.	Coconino ponderosa	79	44	33	11.7
ponderosa var. scopulorum Engelm.	Colorado ponderosa	76	34	34	16.8
ponderosa var. scopulorum Engelm.	Montana ponderosa	80	38	35	11.1
jeffreyi Grev. & Balf.	Jeffrey	10	9	27	14.8
coulteri D. Don.	Coulter	50	41	35	27.6
sabiniana Dougl.	Digger	61	37	34	18.7
radiata D. Don.	Monterey	52	43 14	34	16.7
attenuata Lemm.	knobcone	25	14	31	14.9
	Soft pines				
lambertiana Dougl.	sugar	32	20	34	24.5
monticola Dougl.	western white	36	26	34	11.9
	Hybrid pines			t.	
jeffreyi x ponderosa	Jeffrey x ponderosa (JxP) 1-11	10	12	27	16.1
jeffreyi x ponderosa	Jeffrey x ponderosa (JxP) 5-12	13	14	27	13.0
jeffreyi x ponderosa	Jeffrey x ponderosa (JxP) 1-6	11	4	27	13.8
jeffreyi x coulteri	Jeffrey x Coulter (JxCl)	232	50	14	9.0
jeffreyi x jeffreyi x coulteri	Jeffrey x Jeffrey x Coulter (JxJxCl)	198	40	19	8.9
attenuradiata Stockwell & Righter	knobcone x Monterey (KxM)	25	19	31	22.0

 $<sup>\</sup>frac{1}{2}$  As used by the Institute.  $\frac{1}{2}$  As used in this report.

<sup>3/</sup> Following the grid system of the Institute. 4/ Age of planting, most stock 2-0.

bundle were plugged with lumite screening. This stack of 12 cells was placed in a 150 cc. screw-cap jar with a teflon disk serving as the gasket in the cap.

For <u>D</u>. monticolae and <u>D</u>. jeffreyi the fumigation cell was a 1/4-dram vial. Five of these were bound together constituting a bundle. Ten beetles were assigned to each replicate, and these were placed in the cells of two bundles. The opening of each cell was plugged with lumite screening. Each bundle was placed in a 150 cc. fumigation chamber, two chambers being required to handle a replicate. These two chambers were kept together as a unit throughout the test.

When all beetles were in the fumigation chambers, the test substances were apportioned into individual 3 cc. containers, called resin vials. As soon as a substance was apportioned into one of these vials it was quickly placed in the fumigation jar, and immediately the jar was capped tightly. In tests where a saturated vapor concentration was desired, the test material was apportioned with a 10 cc. pipette. It was calculated that 0.2 cc. of resin or other test substance would weigh about 200 to 300 mg. This was a sufficient amount of material to insure a saturation of resin vapors in the atmosphere within the 150 cc. chamber.

In a few tests an attempt was made to get certain subsaturated vapor concentrations by using microamounts of resin which had been calculated to yield, upon complete vaporization, the desired amount of vapor. The resin was dispensed with a hypodermic syringe, and the amount required obtained by turning a micrometer screw against the plunger of the syringe. A quarter-inch square of teflon was used to remove the droplet of resin which formed on the point of the hypodermic needle. The square of teflon with the droplet was dropped into the resin vial, which was in turn placed in the fumigation chamber.

With a two-man team, the process of apportioning a standard sample of resin, putting it in a fumigation jar, and capping the jar was accomplished in less than 10 seconds. The handling of the microsamples was accomplished in 3 to 5 seconds.

Two or three samples of each resin were run through a series of weighings to determine their original weight, and how much weight they lost during the time in the fumigation chamber. Weights were measured to the nearest 0.1 mg. with a chainomatic, analytical balance. The weight lost was regarded as the weight of the vapor in the chamber. Data from the samples were considered to be representative of a particular resin in a specific test. Average vapor weights are presented below with the other data for each test.

As soon as all replicates of a test were capped and labelled, they were set aside at  $73^{\circ} \pm 2^{\circ}$  F. for the required number of days in the treatment period. The number of replicates for any one treatment in a test varied a bit, depending on the species of beetle, the availability of beetles, and the availability of the materials to be tested. Five

or six replicates were used in a great majority of tests; the exact number is given below for each test.

At the conclusion of the treatment period, the fumigation chambers were uncapped, and all the beetles removed and observed for mortality. Beetles which did not move when agitated were considered to be dead. Records were maintained for individual beetles, although the basic figure used in the analysis was the number of dead beetles in each replicate. After this examination at the end of the treatment period, all of the test insects, still within the cells, were held at the same temperature but in a nonresinous atmosphere, and observed at two-day intervals for subsequent mortality. The period in which these observations were made is referred to as the posttreatment periods in this report. All tests were maintained until mortality became too great to be suitable for analysis.

An untreated control was included in every experiment. It was composed of one set of replicates in capped fumigation chambers without any resin or test material.

## Analysis of Data

The procedure adopted in analyzing the mortality data from this season's tests was to treat each set of observations as an entity. That is, the examination at the end of a treatment was considered one set of observations; the examination two days later was considered another set of observations, etc. The numbers of dead beetles for treatments and replicates were arranged in the block order which existed in the test and F-values calculated for each set of observations (Snedecor, 1946, p. 218).

Duncan's multiple-range testing (Duncan, 1955) was applied to all sets of observations for which a treatment F-value was obtained which was greater than F at the 90 percent confidence level. In each table of results ISD (least significant difference) lines were drawn for both the 95 and 99 percent confidence levels. A line was drawn to connect treatments which do not differ at the designated confidence level; consequently, significantly different treatments do not fall on the same lines. After the ISD lines had been drawn, the number of dead beetles was converted to percent mortality. Finally, a composite table was constructed for each experiment, summarizing by material tested the data on vapor concentration and corresponding beetle mortality for the treatment and posttreatment periods.

#### EXPERIMENTS ON THE TOXICITY OF PINE RESIN VAPORS

From earlier and less extensive work Smith (1961b) formulated the hypothesis that bark beetles of the genus Dendroctonus can tolerate resin vapors of host pines but are unable to tolerate those of nonhost pines. This work also suggested that the beetles are unable to tolerate pine resin vapors of host x nonhost hybrids, although the evidence on

the last point was quite uncertain. One of the basic objectives of the experimental work in 1960 was to test the soundness of this hypothesis by extending the research to additional bark beetles and additional pines and pine hybrids. The fumigant toxicity experiments conducted this year are described below for each of the three species of beetles studied.

#### Dendroctonus monticolae

Ten experiments were made with  $\underline{D}$ .  $\underline{monticolae}$  from June 28 to August 4. The materials tested were resins of host and nonhost pines and pine hybrids at saturation and subsaturation. Most of the pines were hard pines, but two soft pines also were included.

#### A. Resin group I at subsaturation:

Resin group I materials included the resin vapors of (a) host pines ponderosa, Coulter, and sugar; (b) nonhost Jeffrey pine; and (c) a hybrid of a nonhost and a host, Jeffrey x ponderosa. Three tests were run using exposure periods of 5, 7, and 9 days. An attempt was made to create an atmosphere in the fumigation chamber that was about 50 percent saturated with resin vapors. This was accomplished, as previously described, by limiting the amount of fresh resin placed in the chamber to a calculated volume which, when fully vaporized, would produce the desired percent saturation. Erratic results were obtained in securing 50 percent vapor saturation (table 2). The best values were for Jeffrey pine and for the Jeffrey x ponderosa hybrid, and these were the resins of most interest.

The results with host resins tell very little; those with the nonhost Jeffrey pine resin are worth noting. A vapor saturation of 50 percent, or nearly so, was obtained only for the 9-day treatment. For this period mortality increased, but not significantly. During the posttreatment periods, mortality was less for the Jeffrey pine resin than for the others, though again the differences were not significant.

Jeffrey pine resin vapor seemed to have a paralytic effect on the beetle, but this could not be measured. This effect might explain the lower mortality of the beetles during post-treatment periods. At subsaturated concentrations, the paralytic effect was apparently insufficient to kill the beetles but merely reduced their activity. When these beetles were released from the vaporous atmosphere, they were able to live longer, perhaps because they had not used as much of their reserve energy as the beetles which had not been held in a subsaturated atmosphere of Jeffrey pine resin. Partial paralysis of D. monticolae in vapors of Jeffrey x ponderosa resin was frequently observed also; however, the paralysis was not as severe as with Jeffrey pine resin vapors.

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Table 2.--Mortality of adult <u>Dendroctonus monticolae</u> in a subsaturated atmosphere of different pine resin vapors for varying periods of exposure\_\_\_\_\_

	Treatmen	t period	:	Posttreatment periods							
C) i	:	:	:		2-day		•	4-day			
	: Vapor	:	LSD :		•	: LSD	:	:	: LSD		
Resin	: sat.	: Mortality :	5%:1%:	Resin	: Mortality	:5%:1%	: Resin	: Mortality	: 5%:1		
	Percent	Percent			Percent			Percent			
				A. 5-da	y exposure						
Coulter	100	7 1	- 1	Jeffrey	20		JxP 1-6	55	1 1		
Ponderosa		10	1 1	Coulter	25		Jeffrey	62			
JxP 1-6	35	12		JxP 1-6	27		Coulter	63			
Jeffrey	41	12		Control	30		Control	63			
Control	0	20	4	Sugar	40		Ponderosa		-		
Sugar	59	22	1	Ponderosa	45		Sugar	83			
				B. 7-da;	y exposure						
JxP 1-6	44	20		Jeffrey	43						
Jeffrey	36	22		Coulter	52						
Coulter	60	22		Control	52						
Ponderosa		23		Ponderosa	53						
Sugar	88	25		Sugar	60						
Control	0	25		JxP 1-6	60						
				C. 9-da;	y exposure		v.)				
Control	0	38	11.9								
JxP 1-6	50	38									
Ponderosa	33	40									
Coulter	43	45									
Sugar	76	48									
Jeffrey	52	60									

<sup>1/</sup> Six 10-beetle replicates.

Lack of sensitivity of the testing technique to the paralytic effects of vapors might be overcome by adding some quantitative measure of performance, possibly by having the beetle feeding on some material such as bark within the fumigation chamber.

## B. Resin group I at saturation:

These tests were made with the same resins as in the preceding tests; however, the resins were used in sufficient amounts to insure a saturated atmosphere in the 150 cc. chamber. Exposure periods of 3, 5, and 7 days were used.

The resin vapor of Jeffrey pine, a nonhost tree, caused a highly significant increase in mortality over other resins and over the control in all tests (table 3A, B, C). Resin vapors from host trees, ponderosa, Coulter, or sugar pines did not cause a significant increase in mortality over the control. Resin vapors from the Jeffrey x ponderosa hybrid also did not cause a significant increase in mortality.

The treatments appear to have a delayed effect, and this is reflected in posttreatment mortality. Moreover, the effect increases with an increase in the treatment period. To illustrate, the following listing shows the relationship between the period of treatment and the number of changes from nonsignificant to significant mortality at the 2-day posttreatment period:

- (a) 3-day treatment + 2-day posttreatment--no changes
- (b) 5-day treatment + 2-day posttreatment--one change
- (c) 7-day treatment + 2-day posttreatment--three changes

The tendency for a proportionately greater increase in posttreatment mortality with increased period of treatment could indicate: (a) a delayed toxic effect which becomes evident only when the beetle is released from the resin vapor atmosphere, (b) the inability of the beetle to persist in its attack with a continued flow of resin, even from a host pine, (c) a stimulating effect of the resin vapor, which causes the beetle to expend its reserve energy more rapidly and to die more quickly.

# C. Resin group II at saturation:

The resins in the second group of pines tested with this beetle were: (a) western white pine, a host; (b) Digger and Monterey pines, nonhosts; and (c) knobcone x Monterey pine, a hybrid of two nonhosts. Each of the three tests reported in this

Table 3.--Mortality of adult <u>Dendroctonus monticolae</u> in a saturated atmosphere of different pine resin vapors for varying periods of exposure

	Treatme	nt period	. :		2-day	Posttreatmer		4-day	
	:		: LSD :		:	: LSD :		+-uay	: LSI
Resin	: Vapor	: Mortality		Resin	: Mortality	7: 5%:1%:		Mortality	
	Mg.	Percent			Percent			Percent	
				A. 3-da	y exposure1/	1			
oulter	1.6	2	3000	Control	15				
ontrol	0.0	3	18, 201	Coulter	20				
onderosa	1.7	5	COME D	Sugar	27				
TxP 1-6	7.2		7 6	Ponderosa	~				
ugar	1.6	10	1, 1,	JxP 1-6	30	1, 1,			
effrey	19.0	53	1 1	Jeffrey	55	1 1			
				B. 5-da	y exposure1/	1			
onderosa	1.9	10		Control	38	1 10	Coulter	65	1
Control	0.0	12		Coulter	40		JxP 1-6	65	
Coulter	1.7	18		JxP 1-6	40		Control	72	11
dugar	1.8	18	7 5 10	Sugar	48		Sugar	72	
TxP 1-6	9.0	18	1, 1,	Ponderosa	63		Ponderosa	85	١.
Teffrey	22.6	98		Jeffrey	100	ald	Jeffrey	100	
				C. 7-da	y exposure1	'			
Coulter	2.0	25		Control	45	1 1	Control	63	1
Control	0.0	27		Coulter	60		Sugar	82	1
TxP 1-6	7.5	28		JxP 1-6	68		JxP 1-6	85	- 11
Ponderosa	2.0	38	13 1/43	Sugar	70		Coulter	88	- 11
Sugar	1.9	42		Ponderosa	87		Ponderosa	97	- 11
Teffrey	21.9	95	ALLEY !	Jeffrey	95		Jeffrey	100	_ `
				D. 5-da	y exposure1/				
Control	0.0	5		Western W	hite 23	1. 1	Western White	e 57	1
V. White	4.1	5		Control	42		Control	65	
onterey	5.0	8		Monterey	55		Monterey	85	1
				E. 5-da	y exposure2/				
CxM	5.4	20		Control	30		Control	70	1
Control	0.0	23		KxM	67		Monterey	83	1
onterey	5.5	23		Monterey	70		KxM	87	
igger	21.5	83		Digger	83		Digger	93	1
				F. 7-da;	y exposure2/				
Control	0.0	23	1.	Control	63				
lonterey	5.5	57		Monterey	93	1 1			
CxM	5.4	57 63		KxM	97				
igger	21.5	100	.1 .1	Digger	100				
				G. 7-da;	y exposure1/				
ontrol	0.0	32		JxP 5-12	53	1	JxP 5-12	82	1
TxP 5-12	9.2	35		Control	53 57		Control	83	
TxP 1-6	8.3	40		JxP 1-11	67		JxP 1-11	88	
	9.2	42		JxP 1-6	77		JxP 1-6	90	

<sup>1/</sup> Six 10-beetle replicates.

<sup>2/</sup> Three 10-beetle replicates.

group contained resin vapors, at saturation, of two or more of these pines. The treatment period was 5 days for two of the tests and 7 days for the third.

Beetle mortality from western white pine and Monterey pine resin vapors was not significantly different at the end of the 5-day treatment period (table 3D). At the end of the 2-day posttreatment period the mortality for the two pines did not differ significantly from the untreated control, but the mortality from Monterey pine was significantly greater than that from western white pine. For the 4-day posttreatment period the mortality from western white pine and the control did not differ, but that from Monterey pine was significantly greater than either. This was also true at the end of the 6-day posttreatment period.

In the other two tests, the vapor toxicities of the resins of Digger, Monterey and the knobcone x Monterey hybrid pines were compared. In the 5-day treatment, only Digger pine caused significantly greater beetle mortality, but at the end of the 2-day posttreatment period mortalities from all three resins were significantly different from the control (table 3E). With a 7-day treatment, the mortality from Digger was significantly greater than from both knobcone x Monterey, and Monterey pines; and these, in turn, were significantly greater than the control (table 3F). All three resins caused significantly greater mortality than the control at the end of the 2-day posttreatment period.

Two other points are noteworthy. First, Digger and Jeffrey pine resin vapor seemed to have a similar effect on <u>D</u>. monticolae. Perhaps this can be explained by the fact that the heat-fractionated liquid derivatives of Jeffrey pine resin are almost identical to those of Digger pine. Second, the knobcone x Monterey hybrid pine resin vapor caused greater mortality than Monterey, though the difference was never at a significant level. This effect was more pronounced in tests with the other two bark beetles.

# D. Hybrid resins at saturation:

The last experiment with <u>D. monticolae</u> was with resins of the Jeffrey x ponderosa hybrids. It was made to check previous findings, and also to compare three of these hybrids having different parental make-up. The treatment period was for 7 days with the resin vapor at saturation.

No significant differences in toxicity of any of the materials tested were found (table 3G). The lack of significant differences in the entire test suggests that  $\underline{D}$ .  $\underline{monticolae}$  can tolerate host x nonhost hybrids of different parental

composition. Thus, though the nonhost Jeffrey pine resin vapor is toxic to this beetle, the toxic effect is apparently "diluted" to a nontoxic level in the Jeffrey x ponderosa hybrid.

## Dendroctonus jeffreyi

The vapor toxicity of resins to <u>D</u>. <u>jeffreyi</u> was determined in four tests made between August 4 and September 4. Resins from the same groups of pines used with the mountain pine beetle were tested against the Jeffrey pine beetle. The pines were different only in their ecological relationship to the beetle since Jeffrey pine is the sole host of this insect. All tests were for 5 days with resin vapor at saturation. The number of tests was limited by the shortage of beetles.

## A. Resin group I at saturation:

In the test with the pines in resin group I (table 4A) both Coulter and ponderosa pines caused a significantly greater mortality than either the control or Jeffrey pine resin. The other nonhost, sugar pine, and the Jeffrey x ponderosa hybrid caused a nonsignificant increase in mortality over that from Jeffrey pine or the control. At the end of the 2-day posttreatment period, mortality with all three nonhosts and the hybrid was significantly greater than with either the Jeffrey or the control, which did not differ.

Here again, then, the hypothesis that the beetles can tolerate resin vapors of their hosts, but not of their nonhosts, held very well except for sugar pine. However, the latter did cause significantly greater mortality during the 2-day post-treatment period. This was one of a number of instances in which nonhost soft pines did not adversely affect the beetles as consistently or as quickly as nonhost hard pines.

# B. Sugar pine resin at saturation:

A separate test to determine the toxicity of sugar pine resin to <u>D</u>. <u>jeffreyi</u> was made to check the results obtained in the resin group I test. The results (table 4B) show that beetle mortality with sugar pine resin was significantly greater than with the control. These results do not agree with the previous findings in which there was no significant difference between the resin and the control. This seems to be largely due to the untreated mortality, which was 35 percent in the group I test and only 25 percent in this test. It could also be attributed to the greater vapor saturation in this test as compared with the earlier one. The value of the vapor toxicity procedure in determining the host relationship of this beetle to sugar pine will have to remain questionable.

Table 4.--Mortality of adult <u>Dendroctonus jeffreyi</u> in a saturated atmosphere of different pine resin vapors for 5 days

	Treatme	nt period		Posttreatment periods								
	:	:	:		2-day		:		4-day			
	:	:	LSD		:		LSD:		:	: LSD		
Resin	: Vapor	: Mortality	: 5%:1%:	Resin	: Mortality	: 5	%:1%:	Resin	: Mortalit			
	Mg.	Percent			Percent				Percent			
				A. Exper	iment1/							
Jeffrey	21.0	32 35 40		Jeffrey	67		1.					
Control	0.0	35		Control	75	1,	11					
JxP 1-6	9.4		h	Sugar	93							
Sugar	2.3	50		JxP 1-6	97							
Coulter	2.3	62	1	Coulter	97							
Ponderosa	2.7	67	1	Ponderosa	98	- 1	1					
				B. Exper	iment1/							
Control Sugar	0.0	25 53	1, 1,	Control Sugar	48 87	1,	1,	Control Sugar	77 97	1, 1,		
24641	3			ALCOHOLOGICA CONTRACTOR OF THE PARTY OF THE	100							
				C. Exper	iment—							
Digger	20.0	22	1 1	Digger	48							
W. White	4.7	32		Control	62	1.						
Control	0.0	37	200	Western Wh	ite 82							
Monterey	5.6	60		Monterey	95							
KxM	6.9	72		KxM	98		1					
				D ==	2/							
				D. Exper	1ment							
Control	0.0	23		Control	43	1.	1					
JxP 1-6	12.7	30		JxP 1-11	87							
JxP 1-11	12.7	40		JxP 1-6	87							

<sup>1/</sup> Six 10-beetle replicates.

<sup>2/</sup> Three 10-beetle replicates.

## C. Resin group II at saturation:

A 5-day treatment period was used in the test with resins from the four pines, all nonhosts, in resin group II. The summary of data (table 4C) shows (1) a significantly greater mortality for the nonhost Monterey pine and for the hybrid of two nonhosts, knobcone x Monterey pine; (2) no significant increase in the mortality with the other two nonhosts, western white pine and Digger pine. At the 2-day posttreatment period beetle mortality for western white pine was significantly greater than for the control, but for Digger it remained nonsignificant. The effect of Digger pine resin vapor was, again, very similar to that of Jeffrey pine, and this can be explained by the similarity of their heatfractionated volatile derivatives. Mortality with western white pine was very similar to that with sugar pine; it was not significantly greater at the end of the 5-day treatment period, but it was greater at the 2-day posttreatment period.

Why <u>D. jeffreyi</u> is not found on Digger pine in nature is problematical. One possible explanation is that Digger pine occurs at a much lower elevation than the beetle normally does. Perhaps the beetle is unable to survive in the hot, dry climate that Digger pine is able to tolerate. Difficulties in rearing the beetle at low elevations lend support to this explanation. A second possibility, and one which also seems logical, is that resin vapor is not the only factor in beetle-host relationships. Thirdly, it may be that resin has other effects on the beetles than that measured by the technique used in these studies.

# D. Hybrid resins at saturation:

The toxicity of resins from two Jeffrey x ponderosa pine hybrids to <u>D</u>. <u>jeffreyi</u> was compared using trees which had different Jeffrey female parents but the same ponderosa male parent. The results (table 4D) show no significant difference in beetle mortality for the two hybrids and the control at the end of the treatment period. At the end of the 2-day posttreatment period, mortality from neither of the two hybrids was different from the other, but for both it was significantly greater than for the control.

Thus, the results of the tests with hard pine resins against D. jeffreyi very closely follow the stated hypothesis except for Digger pine. The exception may be explained by resin chemistry. Results with both sugar pine and western white indicate the unreliability of applying the hypothesis to soft pines. In general, D. jeffreyi was unable to tolerate hybrid pine resin vapors as well as it tolerated host resin vapors.

#### Dendroctonus brevicomis

Vapor toxicity studies with <u>D</u>. <u>brevicomis</u> were much more extensive than with the other two beetles. They involved 20 tests with resin and resin derivatives of various species of pines. Some of the pines were hosts, and others nonhosts of the beetle. Seventeen tests were with resin vapors at saturation, but three were with resin vapors at subsaturation. Also investigated in this series of tests was the effect of ponderosa pine resin source and age of beetle on vapor toxicity.

## A. Resin group I at subsaturation:

Group I resins tested at subsaturation were from the same species of pines as those used with the other two bark beetles. Ponderosa and Coulter pines are hosts of D. brevicomis; sugar and Jeffrey pines are nonhosts; and the Jeffrey x ponderosa hybrid is a combination of nonhost and host. Three tests were run using treatment periods of 5, 7, and 9 days. Greater success was obtained in securing 50 percent vapor saturation in these tests than in the ones with D. monticolae. The results (table 5) show that Jeffrey pine resin vapor, even at approximately 50 percent saturation, caused a significantly greater beetle mortality than all the other resins or the control. None of the others were significantly different from each other or from the control during the treatment period. Mortality caused by most of the resins was not significantly greater than the control in the posttreatment periods either.

## B. Resin group I at saturation:

The same resins tested at subsaturation in the preceding series of experiments were also tested at saturation with exposure periods of 3, 5, and 7 days. Jeffrey pine resin vapor caused 100 percent beetle mortality in all tests (table 6A, B, C). Since this was highly significant for the shortest treatment period, the data for this resin were not included in the analyses of the other data from these tests. Apparently D. brevicomis was less able than D. monticolae to tolerate the vapors of Jeffrey pine resin. This could be attributable to the larger size of D. monticolae as well as to its broader range of tolerance. Neither ponderosa nor sugar pine resin vapor caused a significant increase in the mortality for any of the three exposure periods. Coulter pine resin vapor caused a significant increase with only the 3-day exposure. The Jeffrey x ponderosa hybrid pine resin vapor caused a significant increase in mortality for both the 3-day and 7-day exposures. All resins tended to cause a significant increase in mortality during the posttreatment periods.

Table 5.--Mortality of adult <u>Dendroctonus brevicomis</u> in a subsaturated atmosphere of different pine resin vapors for varying periods of exposure 1/2

	Treatmen	t period		Posttreatment periods						
	:	: 13.15	:		2-day		:	4-day		
	: Vapor	:	: LSD :		:	: LSD	:		: LSD	
Resin	: sat.	: Mortality	: 5%:1%:	Resin	: Mortality	: 5%:1%	Resin	Mortality	: 5%:1	
	Percent	Percent			Percent			Percent		
				A. 5-day	exposure					
Ponderosa	44	3		JxP 1-6	7	1 1	Coulter	28	1 1	
JxP 1-6	54	3 3 7		Ponderosa	ıi		Ponderosa	29		
Control	0	7		Coulter	14	5	Sugar	32		
Coulter	29	8		Sugar	15		Control	43	72	
Sugar	65	8	l. I.	Control	19	1, 1,	JxP 1-6	7+7+	1, 1	
Jeffrey	66	56		Jeffrey	78	1 1	Jeffrey	90		
		*		B. 7-day	y exposure					
Coulter	48	7	1 1	Coulter	22	1 1	Coulter	53	1. 1	
JxP 1-6	55			JxP 1-6	33		Ponderosa	61		
Ponderosa	48	13 14		Sugar	35		Control	69		
Sugar	100	15		Ponderosa	38		Sugar	71	113	
Control	0	25		Control	40	1. 1.	JxP 1-6	72	1.	
Jeffrey	75	75		Jeffrey	92		Jeffrey	97	1	
				C. 9-day	y exposure					
Control	0	22		Control	46	1 1	Control	76	1. I	
Coulter	48	25		Coulter	49		Coulter	90		
Sugar	100	26		Ponderosa	57		JxP 1-6	90		
Ponderosa	12	28		Sugar	58		Sugar	92		
JxP 1-6	48	33	, ,	JxP 1-6	68	1. 11	Ponderosa	99		
Jeffrey	63	68		Jeffrey	93		Jeffrey	100		

<sup>1/</sup> Six 12-beetle replicates.

Table 6.--Mortality of adult <u>Dendroctonus brevicomis</u> in a saturated atmosphere of different pine resin vapors for varying periods of exposure

	Treatmen:	t period :	. :		2-day	Posttreatm	ent periods	4-day		
	:	:	: LSD :		Mortalit Percent		Resin	Mortality Percent	: LSI : 5%:	
				A. 3-day	exposure1	/	V			
Control Sugar Ponderosa Coulter JxP 1-6 Jeffrey	0.0 1.9 2.4 1.8 8.6 17.7	4 7 11 15 100		Control Ponderosa Sugar JxP 1-6 Coulter	14 21 28 31 35		Control Sugar Coulter JxP 1-6 Ponderosa 2/	50 63 65 72 74	1	
				B. 5-day	exposure	/				
Sugar Control Ponderosa JxP 1-6 Coulter	1.3 0.0 2.5 8.1 1.9	24 26 29 31 39		Control Coulter JxP 1-6 Ponderosa Sugar 2/	46 69 69 71 71		Control Ponderosa Sugar JxP 1-6 Coulter	71 89 89 90 93		-
				C. 7-day	exposurel	/				
Coulter Control Sugar Ponderosa JxP 1-6	2.7 0.0 1.9 2.5 8.6	47 51 63 65 76		Control Coulter Sugar JxP 1-6 Ponderosa 2/	79 83 86 86 92					
				D. 5-day	exposure	4				
Control Sugar JxP 1-6 Coulter JxP 5-12 JxP 1-11 Ponderosa	0.0 2.5 7.7 2.6 8.1 7.7 3.9	11 13 17 17 21 25 31		Control Sugar Coulter JxP 1-6 JxP 5-12 Ponderosa JxP 1-11	24 29 31 42 44 47 49		Control JxP 5-12 Coulter Sugar JxP 1-6 JxP 1-11 Ponderosa	50 65 67 67 69 71 76		
				E. 7-day	exposure1	/				
Control Coulter Ponderosa Sugar JxP 1-11 JxP 1-6	0.0 2.5 3.1 2.3 9.5 8.5	42 54 56 60 67 79	Bell C	Control JxP 1-11 Sugar Coulter JxP 1-6 Ponderosa	74 85 86 93 94 97					
				F. 5-day	exposure 3	<i>'</i>				
Control JxP 1-11 JxP 1-6	0.0 8.5 8.0	18 52 65		Control JxP 1-6 JxP 1-11	43 77 80	1 1	Control JxP 1-6 JxP 1-11	78 95 97	4	
				G. 5-day	exposure	with sugar	pine resin4/			
10 drops 20 drops 5 drops Control	2.7 2.9 2.4 0.0	10 14 14 17		10 drops Control 5 drops 20 drops	44 48 49 51		Control 10 drops 20 drops 5 drops	62 63 70 7 <sup>4</sup>		
				H. 5-day	exposure	with sugar	pine resin <sup>5</sup> /			
1.0 cc Control 1.5 cc	2.7 0.0 2.9	8 11 19		1.0 cc Control 1.5 cc	14 28 42	11	1.0 cc Control 1.5 cc	25 47 61	1	
				I. 5-day	exposure	Lange Control				
W. White Control Monterey KXM Digger	4.7 0.0 5.6 6.9 20.0	33 36 54 57 100		Control Western Whit Monterey KxM 3/	85 92	\	Control Western Whit Monterey KxM 3/	79 88 97 99	'	
- 19	0.0	16		J. 7-day		1 1	0	li C	1	,
Control W. White Cnobcone Monterey KXM	0.0 2.9 4.6 4.9	16 19 26 27 36		Western Whit Control Knobcone Monterey KxM	27 30 43 53 69	111	Control Western Whit Knobcone Monterey KxM	40 43 57 70 83		-

<sup>1/</sup> Six 12-beetle replicates.

<sup>2/ 100</sup> percent mortality, data not used'in calculation of F.

<sup>3/</sup> Five 12-beetle replicates.

<sup>4/</sup> Seven 12-beetle replicates.

<sup>5/</sup> Three 12-beetle replicates.

<sup>6/</sup> Seven 10-beetle replicates.

When the effects of saturated and subsaturated ponderosa pine resin vapors on the beetles during the posttreatment periods were compared, it was found that saturated vapors usually caused greater mortality and the subsaturated vapors less mortality than the controls. Previous work by the author has shown that variations in vapor concentrations of ponderosa pine resin had no effect on the mortality rate of a given brood of <u>D. brevicomis</u>. Therefore, the differences observed in these two series of tests may be an expression of a difference between the two broods of beetles. This possibility will be pursued in a later section of this report.

# C. Resin group I and hybrids at saturation:

Tests of the vapor toxicity of resins from some of the pines and from certain hybrids were repeated with <u>D. brevicomis</u> primarily to check previous results. Treatment periods were 5 and 7 days with saturated resin vapors. In both tests Jeffrey pine was omitted, and one or two different Jeffrey x ponderosa hybrids were included. The source of beetles was different for each of the two tests, and neither brood was the same as for previous tests with resin group I at saturation.

As shown by the data (table 6D and E) ponderosa, Coulter, and sugar pines caused no significant increase in beetle mortality during the treatment period. These findings were the same as those in the original tests. The two hybrids common to this experiment, Jeffrey x ponderosa 1-11 and 1-6, caused no significant increase in mortality with the 5-day treatment, but both caused a significantly greater mortality than the control with the 7-day treatment. Mortality for the post-treatment periods was not consistent except for the tendency, as in the original tests, for all resins to cause greater mortality than the control. Sometimes these differences were significant, and sometimes they were not.

Resins from the two Jeffrey x ponderosa hybrids were retested using a 5-day exposure period. The data on beetle mortality (table 6F) show the two hybrids to be significantly different from each other and from the control. This was the first time a significant difference was found between the two hybrids; in other tests, differences occurred but not at significant levels. If the difference in this case is real, it is probably due to the two Jeffrey parents, since the ponderosa pine was a common parent to both hybrids. A more sensitive testing procedure might prove this point more satisfactorily. The detection of within-species variation would be a valuable tool for the geneticists in selecting breeding stock.

Two additional tests were made on the vapor toxicity to D. brevicomis of sugar pine resin at saturation. Using a 5-day treatment period, the volume of fresh resin was varied to see if differences in vapor saturation could be obtained, and if so, whether such differences would be reflected in mortality. The summary of the results (table 6G and H) shows no differences in vapor saturation for the different volumes of resin used. Likewise, the mortality data show no significant differences between the various volumes of resin and the control for the treatment periods. Some significant differences appeared during the 4-day posttreatment period, but they were not consistent.

As a whole the results of these tests with sugar pine agree with previous findings showing no significant effect of the resin vapor on <u>D</u>. <u>brevicomis</u>, except during posttreatment periods. These results, together with those with <u>D</u>. <u>jeffreyi</u>, indicate that the basic hypothesis, that bark beetles can tolerate saturated vapors of host resins but cannot tolerate those of nonhost resins, is inapplicable to sugar pine. Since tests with western white pine, another soft pine, produced similar results, the basic hypothesis apparently does not hold for the soft pines.

## D. Resin group II at saturation:

The resins in group II were tested at saturation with  $\underline{D}$ . brevicomis the same as they were with the other two bark beetles. None of the pines in this group are hosts of  $\underline{D}$ . brevicomis. The standard fumigation cells for this species were used with a 5-day treatment; but the larger fumigation cells, normally used for  $\underline{D}$ . monticolae and  $\underline{D}$ . jeffreyi, were used with a 7-day treatment.

With a 5-day treatment Digger pine resin caused a significantly greater beetle mortality than either Monterey pine or the knobcone x Monterey hybrid (table 6I). Mortalities from the last two were, in turn, significantly greater than for western white pine or the control. The effect of Digger pine resin vapor was very similar to that of Jeffrey pine, and the reaction of D. brevicomis was quite similar to the reaction of D. monticolae. Western white pine failed to produce a significant effect. Here again, results of a test with a soft pine failed to support the basic hypothesis. Knobcone x Monterey resin vapor caused greater mortality than Monterey. This is one of a series of instances where the resin of a hybrid of two nonhost trees apparently was more toxic to the beetle than either parent tree.

The results of the 7-day treatment, which was with a different brood of beetles, did not follow the pattern anticipated (table 6J). There were no significant differences in beetle

mortality for any of the resins tested. This is the one test which cannot be explained adequately, since significant differences had been found for a shorter treatment period. The apparent difference in mortality between the control and the knobcone x Monterey is not significant. Differences in the brood and in the type of fumigation cell seem to offer the only explanation for the lack of anticipated results. There is no question about the difference in brood; the beetle mortality in the controls was 36 percent for 5 days for the brood used in the 5-day treatment, but only 16 percent for 7 days for the one used in the 7-day treatment. During the posttreatment periods in the 7-day treatment, mortality caused by both Monterey pine and the knobcone x Monterey hybrid did become significantly greater than the control. The position of western white pine remained the same in both tests, and the relative position of the knobcone x Monterey hybrid remained the same with respect to the two parent trees. The results of these tests again present the possibility of variation in D. brevicomis broods.

## E. Hybrids and resin derivatives at saturation:

Four tests with  $\underline{D}$ . brevicomis were made to determine the vapor toxicity of resin and resin derivatives of ponderosa pine and of the resin of various hybrids. The hybrids were of Jeffrey, Coulter, and ponderosa pines. The materials in all tests were used at saturation.

The first test in this group included resin from a Jeffrey x ponderosa hybrid and a Jeffrey x Coulter hybrid, and turpentine derived from ponderosa pine resin by heat fractionation. The turpentine had been fractionated in 1958 by N. T. Mirov, from fresh resin of a ponderosa pine other than the one used in this group of tests. The vapor toxicity of resin and turpentine from this same tree had been tested previously in 1958, and the results (Smith, 1961b) were quite similar to those obtained this year. The treatment period was 5 days.

As shown by the data (table 7A) there was no significant difference in beetle mortality between the two hybrids or between them and the control. A significant difference did show up between turpentine and the other three treatments. There was no relative change during the posttreatment periods. These findings conclusively show that the vapors of turpentine are not at all similar to the vapors of fresh resin, either in their effect on the beetle or in their vapor saturation point. Ponderosa pine turpentine saturates the atmosphere of a 150 cc. fumigation jar at over 8.0 mg., while fresh resin of this tree saturates it at between 2.0 and 3.0 mg.

Table 7.--Mortality of adult <u>Dendroctonus brevicomis</u> in a saturated atmosphere of the vapors of pine hybrid resins and ponderosa pine resin derivatives and sources for varying periods of time

	Treatme	ent period	:	Posttreatment periods						
:		In The FF	: :		2-day				4-day	
Resin	Vapor Mg.	: Mortality Percent	: LSD : 5%:1%:	Resin : 1	Mortalit	: LS : 5%		Resin :	Mortalit Percent	: LSD : 5%:1
	<u></u> 6.	rereens		A. 5-day e		/			rerection	<u>:</u>
Control JxP 1-6 JxCl Turpentine	0.0 7.2 9.5 8.5	17 17 28 50		JxP 1-6 Control JxCl Turpentine	25 31 42 83	1		Control JxP 1-6 JxCl Turpentine	61 69 69 100	
				B. 5-day ex	xposure	/				
Control JxP 1-11 JxJxC1	0.0 8.9 14.3	21 36 100	' <sub>1,</sub> 1,	Control JxP 1-11 2/	42 57	1	1	Control JxP 1-11	61 75	
				C. 7-day ex	xposure3	/				
Control Pond. resin Pond. super JxP 1-6 JxP 1-11	0 0	16 21 30 33 44		Control Pond. resin Pond. super. JxP 1-6 JxP 1-11	27 46 61 64 70	1		Control JxP 1-6 Pond. resin JxP 1-11 Pond. super.	144 80 81 86 90	
				D. 7-day e	xposure4	/				
Control 4 wk. super 4 day super Fresh resin Turpentine	r. 2.7	33 35 37 38 95		Control Fresh resin 4 day super. 4 wk. super. Turpentine	47 62 68 78 100			Control 4 wk. super. Fresh resin 4 day super. 2/	98	
				E. 7-day e	xposure5	/				
Control California Cochise Montana Washington Coconino Colorado	0.0 3.1 5.1 3.1 4.0 4.2 3.2	13 29 35 35 36 39 42		Control California Cochise Coconino Colorado Washington Montana	32 64 68 69 69 72 79			Control Cochise California Colorado Washington Montana Coconino	64 89 92 93 93 97 99	

<sup>1/</sup> Three 12-beetle replicates.

<sup>2/ 100</sup> percent mortality, data not used in calculating F.

<sup>3/</sup> Seven 10-beetle replicates.

<sup>4/</sup> Six 10-beetle replicates.

<sup>5/</sup> Six 12-beetle replicates.

In the second test, resins from a Jeffrey x ponderosa hybrid pine and a backcross of a natural Jeffrey x Coulter to Jeffrey were used with a treatment period of 5 days. Beetle mortality in this test (table 7B) was significantly different between the hybrids and between them and the control. The 100 percent mortality caused by the resin vapor of the backcross resembles the effect of Jeffrey pine resin vapor on this beetle. The proportion of Jeffrey in the natural Jeffrey x Coulter hybrid is not known. The vapor saturation of the backcross is about half way between Jeffrey and the Jeffrey x Coulter derived by controlled pollination. Thus, the natural Jeffrey x Coulter hybrid seems to have a 1 to 1 representation of the two parent species.

The third test in this group was a 7-day exposure to the resin vapors of two Jeffrey x ponderosa pine hybrids, ponderosa resin, and the supernatant liquid of ponderosa resin. The supernatant liquid was obtained by holding fresh resin in a closed container at room temperature for a few days. Under these conditions the resin separates into two fractions, one of which crystallizes and settles to the bottom of the container, while the other, a liquid, collects on top of the crystals. The supernatant liquid is much more fluid than whole fresh resin. The results (table 7C) show that fresh resin did not differ from its supernatant liquid and that one of the hybrids caused a significantly greater beetle mortality than the fresh resin or the control. The other hybrid did not. The data indicate that the two hybrids did not differ significantly from each other; however, as in most previous tests, one caused greater mortality than the other. Mortality during the posttreatment periods was significantly different from the control for all the materials tested.

In the fourth and last test of this series, a 7-day treatment period was used to compare the vapor toxicity of ponderosa pine resin with that of some of its derivatives: 4-day-old supernatant liquid, 4-week-old supernatant liquid, and turpentine obtained by heat fractionation at about 165° C. In this test (table 7D) turpentine caused a highly significant increase in beetle mortality compared to fresh resin and the supernatant liquids; mortalities for the other three materials were not significantly different from each other or from the control. In the posttreatment periods all materials differed significantly from the control. The vapor saturation was about the same for fresh resin and the supernatant liquids, but that of turpentine was much greater.

Resin vapors of the Jeffrey x ponderosa pine hybrids in these four tests usually caused a significantly greater beetle mortality than the control. The resin of the backcross hybrid (J x J x Cl) performed much like Jeffrey pine resin, while the Jeffrey x Coulter hybrid resin seemed much like that of Jeffrey x ponderosa, though the single test with the former probably does not warrant a conclusion. The supernatant liquids of ponderosa were quite similar in toxicity to fresh resin, but turpentine was distinctly different.

#### F. Ponderosa pine resin sources at saturation:

The vapor toxicity of resins from six geographic sources of ponderosa pine was determined in another test with D. brevicomis. The six sources were chosen to represent the very extensive range of this tree, since differences in the resin have been found among ponderosa pines from different parts of the range. These differences seem to be caused by variations in the proportions, rather than in the kinds of molecules obtained by heat fractionation. The purpose of the test was to see if such differences would have an effect on the beetle which could be detected with the vapor toxicity technique. If such differences were found, they might help explain the absence of the beetle in many parts of the range of the tree. The resins were tested at saturation with a 7-day treatment, and all resins were collected from trees grown at the Institute from seed gathered at the source.

There were no significant differences at the 5 percent level in beetle mortality among any of the sources tested, but all caused a significantly greater mortality than the control (table 7E). At the 1 percent level the resin from the California source did not differ either from the control or from any of the other sources; but the other five sources all differed significantly from the control. Large differences occurred in the vapor saturation of the six resins, but these differences were not reflected in the mortality. The resins with approximately the same vapor saturation (California, 3.1 mg., Montana, 3.1 mg., and Colorado, 3.2 mg.) completely bracketed the range of mortality.

The effect of all sources of resin was quite similar in the posttreatment periods.

# G. Ponderosa pine resin and age of beetle:

Two tests were made to determine how beetles of different ages were affected by resin vapors. D. brevicomis was the test insect and saturated vapors of ponderosa pine resin the test material.

In the first test, beetles of two different ages with respect to time of emergence were tested using treatment periods of 3 and 5 days. All beetles were collected from the brood material at the same time and were held at 73° ± 2° F. before, during, and after treatment; i.e., the entire test was set up according to the standard procedure. Then one-half of the replicates were set aside, outside fumigation chambers, for 3 days, while the other half of the replicates were put into the fumigation chambers. The replicates treated immediately are considered the O-day age class, while those treated 3 days after emergence are the 3-day age class. The replicates that had been set aside for 3 days were placed in fumigation chambers at the end of that time and treated the same as the preceding group. Examinations were made at 3, 5, 6, and 8 days. Only the 8-day examination included all of the replicates since at the shorter periods some of them were still under test.

One meaningful way to interpret the analyses (table 8A) is to compare sets of replicates of beetles of different ages but with identical treatments. In six such possible comparisons in table 8A there were no significant differences in mortality attributable to age. For example, the mortality of 0-day and 3-day-old beetles with a 3-day treatment period was 47 percent and 46 percent respectively at the 6-day observational period. The mortality of 0-day and 3-day-old beetles with a 5-day treatment was 67 percent and 57 percent respectively at the 8-day observational period. This strongly indicates that, with beetles of comparable age, time elapsed after emergence (up to 3 days) is not an important factor affecting the vapor toxicity of resin.

The second test in this series was much the same as the first except a 5-day treatment with 0-, 2-, and 4-day-old beetles was used. Again the entire test was set up at one time, with one-third of the replicates placed in the fumigation chambers on each of the assigned days.

The results (table 8B) clearly show that no significant differences can be associated with vapor toxicity of resin and time elapsed after emergence for either treated or untreated sets of replicates. The significant differences in the table are those which have occurred in other tests during the posttreatment period.

Thus, in both tests time elapsed after beetle emergence does not appear to be a critical factor in the technique, so long as the beetles used in a test are of comparable ages.

Table 8.--Effect of beetle age of <u>Dendroctonus brevicomis</u> on results of vapor toxicity tests with saturated vapors of ponderosa pine resin\_

Unit			: LSD : :5%:1%:Unit		: LSD : :5%:1%:Unit		: LSD : : 5%:1%:Unit	: : LSD :Mortality : 5%: 1%
	Mg.	Percent	A. 3- and 5-	Percent day exposur	e with 0- and	Percent 3-day-old b	peetles	Percent
	At 3 days	<u> </u>	At	5 days	At	6 days	At	8 days
0-C-3 <sup>2</sup> / 3-T-5 3-C-5 3-T-3 0-T-3 3-C-3 0-C-5 0-T-5	0.0 2.9 0.0 3.4 2.5 0.0 0.0 2.8	4 4 7 8 10	0-C-5 0-T-5 0-C-3 0-T-3	14 19 21 42	0-C-3 3-C-3 3-T-3 0-T-3	36 46	3-C-5 3-C-3 0-C-5 3-T-5 0-C-3 0-T-5 3-T-3 0-T-3	53 56 57 61 67 72
			B. 5-day exp	osure with	0-, 2-, and 4-	day-old bee	etles	
	At 5 days	3_	At	7 days	At	9 days	At	ll days
0-T <sup>3</sup> / 0-C 2-T 4-T	3.0 0.0 2.9 2.9	13 20	0-C 2-C 2-T 0-T	30 30 35 40	O-C 2-C 4-C O-T 2-T 4-T	55 58 62 82 83 87	4-C O-C 2-C 2-T 4-T O-T	83 85 95 98 98 100

<sup>1/</sup> Six 12-beetle replicates.

<sup>2/</sup> Entries in "Unit" column coded for age of beetle, type of treatment, and days in fumigation chamber; example O-C-3:

<sup>0 =</sup> Age of beetle in days since emergence.

C = Check, without resin; T = treated, with resin.

<sup>3 =</sup> Days in fumigation chamber.

<sup>3/</sup> Entries in "Unit" column coded for age of beetle and type of treatment; example 0-T:

<sup>0 =</sup> Age of beetle in days since emergence.

T = Treated, with resin; C = check, without resin.

#### COMPARISONS OF BEETLES

#### Comparisons among species

The major conclusion derived from the experiments described above is that each species of beetle tested is affected quite differently by the vapors of various pine resins; and this response, as measured by adult mortality in an atmosphere of saturated resin vapor, is closely associated with the host relationship between the beetle and pine species. The beetles differ, too, in their range of tolerance to nonhost resin vapors, and this tolerance seems to be related to the degree of host specificity. D. monticolae, a polyphagous species, seems to tolerate nonhost and hybrid resin vapors better than the oligophagous D. brevicomis and the monophagous D. jeffreyi (table 9).

Table 9.--Tolerance of three species of <u>Dendroctonus</u> to nonhost and hybrid resin vapors

	0 0	Reaction1/						
Material tested		monticolae	0	brevicomis	0	jeffreyi		
Jeffrey x ponderosa hybrid resin at saturation		0		*		*		
Monterey pine resin with 5-day treatment		o ·		*		*		
Sugar pine resin during treat- ment		-		0		*		
Western white pine resin during treatment and posttreatment		-		0		*		

Entries in body of table signify the following:

## Comparisons within a species

Tests with <u>D</u>. <u>brevicomis</u> were conducted with beetles of more than one brood or source as described earlier in this report. Therefore, it was possible to look for differences between broods, since each brood was represented by at least one set of six replicates of 12 beetles each in the controls for the 5-day treatments. When the data for the different broods were grouped and subjected to an analysis of variance, a highly significant F-value was obtained for the treatment period. There was a significant difference in

<sup>\*</sup> Significant increase in mortality; o No significant increase in mortality; - Host of beetle, therefore not applicable.

beetle mortality between brood #2 and broods #1 and #3 (table 10). There was no difference between three different collections of brood #4. These general relationships held during the posttreatment periods.

The difference between broods #1 and #2 is illustrated further by the mortality data for 5- and 7-day treatments of beetles with ponderosa pine resin vapor (figure 3 and 4). The treatments may not have been quite comparable, since the tests with brood #1 were with vapor at saturation, while those with brood #2 were with vapor at 44 percent saturation. However, variations in the percent saturation of ponderosa pine resin vapor have not been found to affect the mortality rate of D. brevicomis. The mortality rate of treated beetles of brood #1 was greater than the untreated, while the mortality rate of treated beetles of brood #2 was less than untreated for both periods of exposure. Though the differences within a brood are not statistically significant, the data suggest that "low vigor" beetles (brood #1) cannot tolerate ponderosa pine resin vapors as well as "high vigor" beetles (brood #2).

#### COMPARISONS OF RESINS

In the course of the experiments, it was possible to obtain a reasonably accurate measure of three properties of the resins and resin derivatives by continued weighing of the test resin samples. These three properties are: (1) vapor saturation at  $73^{\circ} \pm 2^{\circ}$  F. in the 150 cc. fumigation chamber, (2) percent volatility at  $75^{\circ} \pm 3^{\circ}$  F. in unsealed containers for 7 days, (3) percent volatility with certain conditions of time and temperature.

## Vapor saturation

Vapor saturation as used in this study is defined as the weight of vapor necessary to saturate the 150 cc. volume of the sealed fumigation chamber. It was determined by serial weighings during the course of the experiments. These weighings showed that with each of the various materials used in the tests the atmosphere within the chamber became saturated within 24 hours. Figure 5 shows the weights of resin vapor of the various pine species and hybrids required to saturate the 150 cc. volume. The values range from about 2 mg. for sugar and Coulter pines to about 20 mg. for Jeffrey and Digger pines. The range of values for one tree may be partially an expression of experimental error, though it is thought to be caused by seasonal variation in resin. No general association between vapor saturation and the effect of the resin vapor on a given species of bark beetle was observed.

Weights of vapors of resin sources and resin derivatives of ponderosa pine required to saturate are plotted in figure 6. Within this species, the values ranged from about 2.5 mg. for the California source to about 5.0 mg. for the Cochise, Arizona source. The two

Table 10.--Mortality of different broods of  $\underline{\underline{Dendroctonus}}$   $\underline{\underline{brevicomis}}$  in the controls for 5-day treatments.  $\underline{\underline{l}}'$ 

Treatment period			: Posttreatment periods						
	: :	:		2-day	:		4-day	-	
Brood No.	: Mortality : Percent	ISD : 5% : 1% :	Brood No.	: Mortality : Percent	ISD : 5% : 1% :	Brood No.	: Mortality : Percent	ISD 5%: 1	
2	7		2	19		2	43		
4-a	11		4-a	24		4-a	50		
4-b	19		4-с	42		4-c	61		
4-c	21		1	46		4-b	63	11,1	
1	26		4-ъ	49		1	71		
3	36		3	53		3	79		

1/ Six 12-beetle replicates.

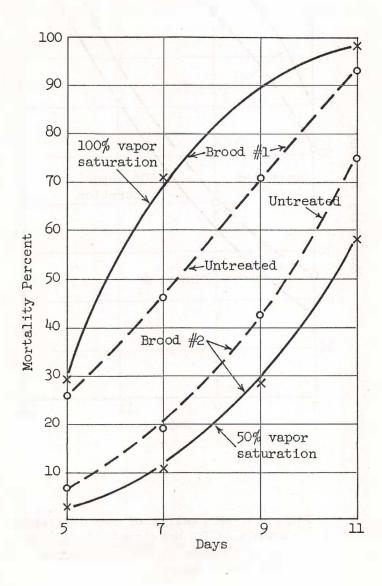


Figure 3.--Mortality of two different broods of <u>D</u>. <u>brevicomis</u> following 5-day treatments with ponderosa pine resin vapor.

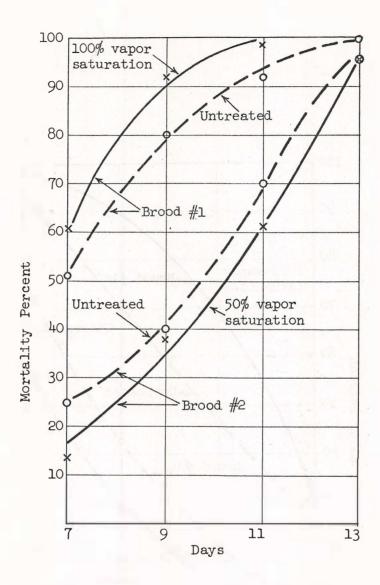


Figure 4.--Mortality of two different broods of <u>D</u>. brevicomis following 7-day treatments with ponderosa pine resin vapor.

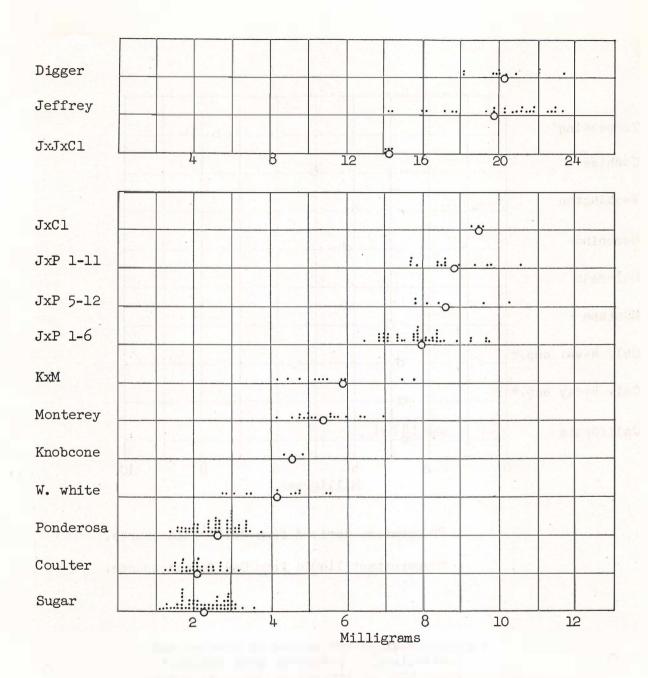
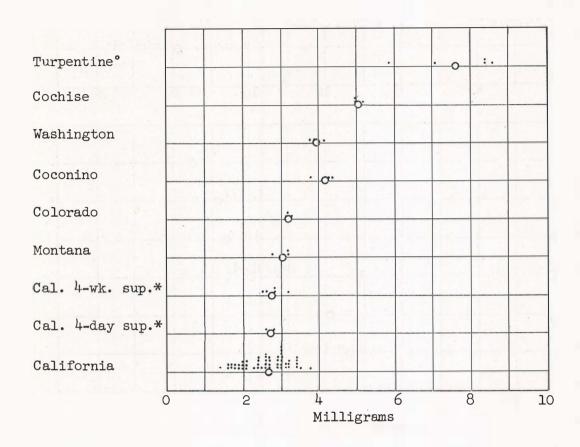


Figure 5.--Vapor saturation of pine resins at 73° ± 2°F. in 150 cc. volume. Each dot is one sample; o is mean value for the species indicated.



°Turpentine derived from California source.

Figure 6.--Vapor saturation of sources and derivatives of ponderosa pine resin at 73° ± 2°F. in 150 cc. volume. Each dot is one sample; o is the mean value for the source indicated.

<sup>\*</sup>Supernatant liquid from California source.

natural derivatives of ponderosa resin, the 4-day and 4-week supernatant liquids, were almost identical with the resin, while turpentine, the heat-fractionated derivative, was distinctly greater in vapor saturation.

Weights of ponderosa pine resin vapor required to saturate the 150 cc. fumigation chamber at different times during the summer are plotted in figure 7. All the data are for resin from the single tree used in all tests. Though the degree or two change in temperature during the course of the experiments could account for slight changes in vapor saturation, it cannot account for the very appreciable increase in vapor saturation from late June to early September. Though an increase in the relative proportions of resin constituents could account for some of this change in vapor saturation, it is quite likely that changes in the quality of resins could also be responsible.

# Percent volatility at 75° F.

Information was obtained on the percent volatility of the resin samples after they had been removed from fumigation chambers. The original sample size was 200 to 400 mg. At the conclusion of a test and after the samples had been weighed again, they were placed in a room at 75° ± 3° F. and allowed to vaporize for about 7 days in most cases. It was found that within 2 or 3 days under these conditions the weight of the samples changed very little. The range in percent volatility of the resins of the species and hybrids tested was appreciable (figure 8). At one extreme is sugar pine with a percent volatility of about 2 percent, at the other is Monterey pine with a percent volatility of 25 percent.

The volatility of resin sources and resin derivatives of ponderosa pine ranged from about 19 percent for the Montana resin to almost 31 percent for the Arizona (figure 9). The turpentine was much more volatile. In general, there is little correlation between the percent volatility and the effect of the resin vapor on adult beetles. This again points to the quality rather than the quantity of resin vapor as the important factor in vapor toxicity.

### Percent volatility with increased temperature

The vapor saturation and percent volatility of resin at room temperature are expressions of the properties which could be important in vapor toxicity tests. Percent volatility at elevated temperatures probably bears little relation to toxicity. Nevertheless, since the opportunity was available, many of the resin samples were subjected to increased temperatures for specified periods of time in a standard laboratory oven to see the effect of higher temperatures on volatility. The temperature-time conditions were (a) 50° C. for 40 hours, (b) 60° C. for 48 hours and then for an additional 72 hours, (c) 80° C. for 72 hours, (d) 100° C. for 48 hours. Since the additional 72 hours at 60° C. did not alter the weight losses to any appreciable amount, the two periods at 60° C. are presented together as 120-hour period.

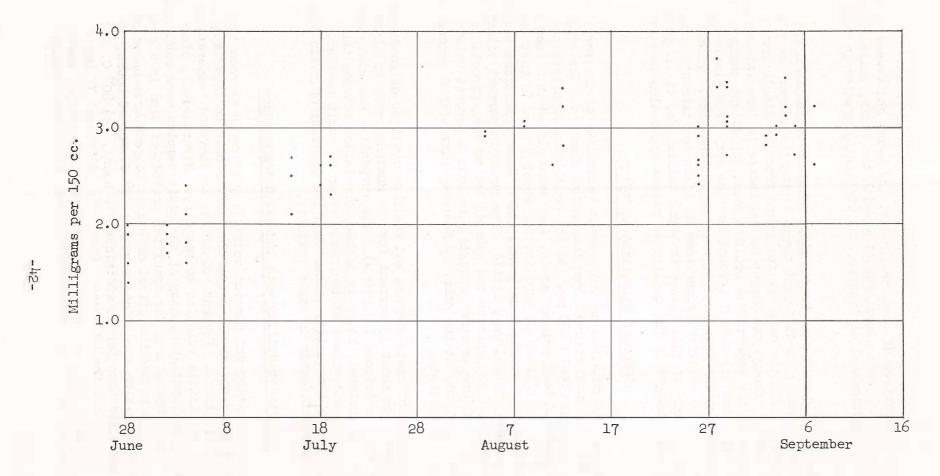


Figure 7.--Seasonal variation in vapor saturation of ponderosa pine resin at  $73^{\circ}F$ .  $\pm$   $2^{\circ}$  in 150 cc. volume. Each dot represents one sample.

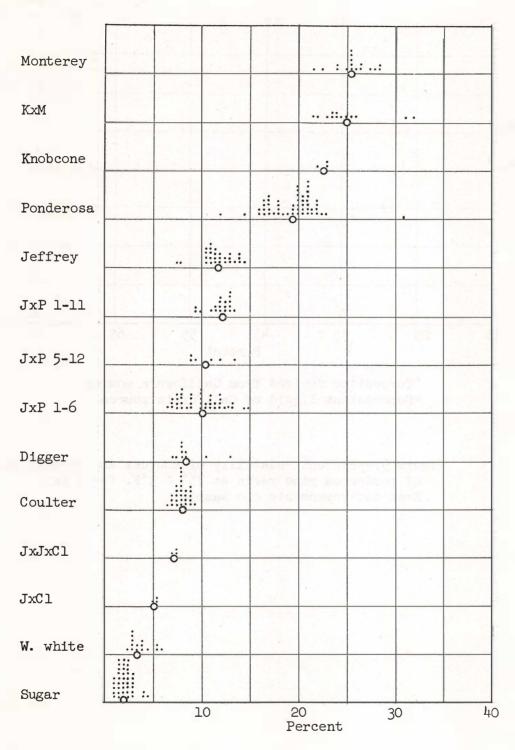
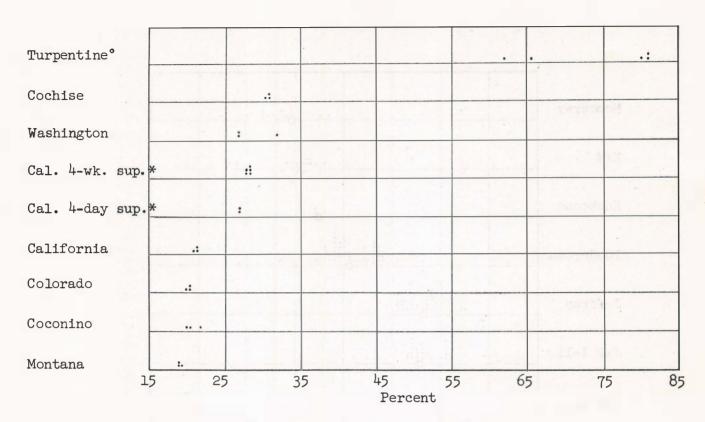


Figure 8.--Percent volatility of pine resins at 75° ± 3°F. for 7 days. Each dot is one sample; o is mean value for the species indicated.



<sup>°</sup>Turpentine derived from California source. \*Supernatant liquid of California source.

Figure 9.--Percent volatility of sources and derivatives of ponderosa pine resin at 75° ± 3°F. for 7 days. Each dot represents one sample.

The values obtained were very consistent for one set of samples of a resin and were surprisingly consistent for all samples of a given species. The percent volatility at  $100^{\circ}$  C. ranged widely among the species, from 35 percent for Monterey to 12 percent for J x J x Cl, and was usually much greater than that given by Mirov (1961) for distillations at temperatures above  $100^{\circ}$  C. (figures 10 and 11). There appears to be a tendency for the volatility of a hybrid resin to be less than that of either parent.

### CONCLUSIONS

This year's studies have provided a clearer insight into the beetle-host relationships of certain <u>Dendroctonus</u> species and various pines and pine hybrids. They have added to our knowledge about the vapor toxicity of pine resins to the beetles. Finally, they have yielded new information on two characteristics of the resins of several pines; namely, their vapor saturation and percent volatility. An attempt has been made to summarize in the accompanying chart (figure 12) knowledge accrued from these three facets of the research.

From these results of the studies it may be concluded:

- (1) The hypothesis that bark beetles can tolerate saturated resin vapors of host pines but cannot tolerate those of nonhost pines holds for the hard pines, but not the soft pines. This may indicate that resin quality is an important factor in the resistance of hard pines to bark beetles.
- (2) The fact that the hypothesis did not hold for soft pines suggests (a) that resins of soft pines differ from those of hard pines and (b) that, if resin quality is a factor in host resistance of soft pines, a more sensitive testing procedure is required to demonstrate it.
- (3) The ability of a bark beetle to tolerate nonhost resin vapor is associated with its host specificity. Polyphagous species are more tolerant of nonhost resins than oligophagous species, while monophagous species are more intolerant of nonhost resins than oligophagous species.
- (4) The toxicity of two nonhost pines is transmitted to the hybrid of the two. The variable results achieved with host x nonhost pine hybrids suggest that in the hybrid the toxic properties of the nonhost pine may or may not be "diluted" to a nontoxic level by the nontoxic properties of the host pine.

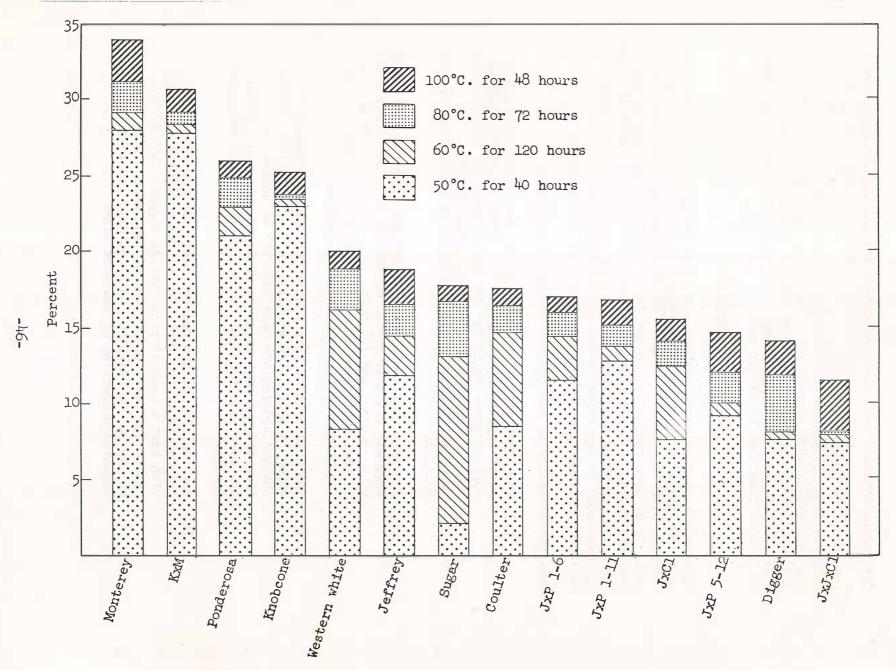


Figure 10. -- Percent volatility of pine resins with temperature and time.

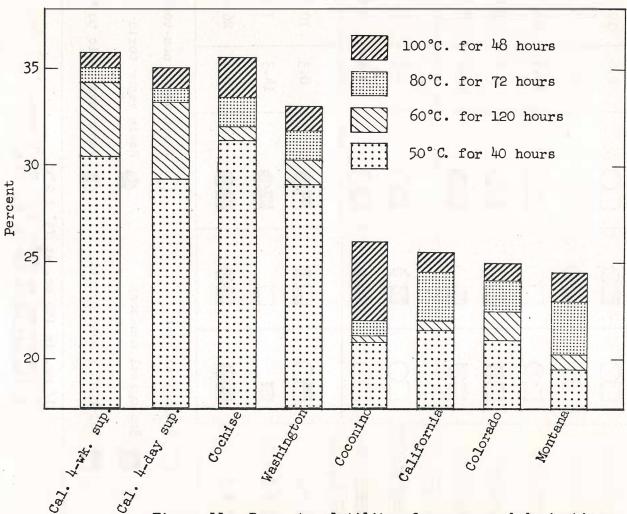


Figure 11.--Percent volatility of sources and derivatives of ponderosa pine with temperature and time.

	]	Dendroctonus	Resin Cha	Resin Characteristics			
Pinus	monticolae	jeffreyi	brevicomis	Vapor satur.	Percent volatility		
hard pines				1/	2/	3/	
ponderosa				2.6	19.3	25.4	
<u>coulteri</u>				2.1	8.1	17.6	
jeffreyi							
radiata				5.4	25.6	33.8	
sabiniana				20.4	8.8	14.0	
soft pines lambertiana	ГО			2.3	2.1	17.6	
monticola				4.2	3.6	19.8	
hybrid pines	T I						
<u>1 x p</u>	<b>B</b> O		ВО	8.3	10.0	17.1	
<u>j</u> x <u>j</u> x <u>cl</u>			EO	14.3	7.4	11.5	
axr				6.1	26.0	30.6	
Ec	cological hose cological non-	-host	Resi	vapor non vapor to	xic		
	2/ Unconfin	150 cc. at 7 ned at 75° ± ned at 100°C	3° ± 2°F. 3°F. for 7 da . for 48 hours	ays.			

Figure 12.--Resin characteristics for certain pines, and insecthost relationships between the pines and three <u>Dendroctonus</u> species, based on host records and toxicity of <u>saturated</u> resin vapors.

- (5) The vapor toxicity of pine resin is more attributable to quality than to the quantity of resin required to produce saturation.
- (6) The absence of <u>D. brevicomis</u> on <u>P. ponderosa</u> in parts of the range of the tree cannot be explained by the vapor toxicity of their resins.
- (7) Differences in the effects of vapors of fresh resin and turpentine indicate the loss or change of basic molecular constituents in the process of heat fractionation. Such differences do not occur between fresh resin and its supernatant liquid obtained at room temperature.
- (8) Seasonal variations in resin quality of P. ponderosa and in the vitality of D. brevicomis occur. These variations could help to account for differences that have been noted from time to time in the destructiveness of this beetle.
  - (9) The vapor saturation weights of hybrid resins is usually intermediate between that of the two parents. However, the percent volatility of the hybrid resin is usually less than that of either parent.
  - (10) The manner in which resin actually functions in resisting beetle attack cannot be fully explained by the results of funigant toxicity studies completed to date. Both death and inhibiting paralysis to the beetle are strongly suggested. Inducing abnormal behavior after the beetle reaches the tree and repelling the beetle before or after it reaches the tree are two possibilities which have not been investigated.

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